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Artificial diet and rearing methods for *Thanasimus dubius* (Coleoptera: Cleridae), a predator of bark beetles (Coleoptera: Scolytidae)

John D. Reeve,[a,*] M. Guadalupe Rojas,[b] and Juan A. Morales-Ramos[b]

[a] *Department of Zoology, Southern Illinois University, Carbondale, IL 62901, USA*
[b] *Formosan Subterranean Termite Research Unit, Southern Regional Research Center, USDA-ARS, New Orleans, LA 70124, USA*

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Abstract

Clerid beetles are common natural enemies of bark beetles, and could potentially be used as biological control agents if they could be reared in sufficient numbers. We developed an artificial diet devoid of insect components for rearing *Thanasimus dubius* (Fabricius), a clerid that attacks several economically important bark beetles in eastern North America. We reared larvae of this predator using the artificial diet, and then used either natural or factitious prey to feed the adults so produced. Two different methods of presenting the diet were also examined. We then compared the performance of *T. dubius* reared on the artificial diet with newly-emerged wild individuals collected from the field. Our results suggest that adult predators reared on the diet are near in quality to wild ones, and high R_0 values can be obtained. No difference in prey preference was found between wild and diet-reared individuals after five generations in the laboratory. Sufficient numbers of predators could be generated using these techniques to permit limited field trials of augmentative biological control.

Keywords: Artificial diet; Biological control; *Thanasimus dubius*; *Dendroctonus frontalis*; *Ips grandicollis*; *Callosobruchus maculatus*

1. Introduction

Bark beetles are important pests of coniferous forests throughout the world. They can cause significant economic losses in forests managed for timber production, and are also agents of natural disturbance in many ecosystems (Christiansen and Bakke, 1988; Price et al., 1992; Raffa, 1988; Schowalter et al., 1981). A large community of natural enemies is associated with most bark beetle species, including clerid beetles (Coleoptera: Cleridae) that are predators on both larval and adult bark beetles (Dahlsten, 1982; Mills, 1985; Moeck and Safranyik, 1984). Clerids appear to generate substantial mortality in some systems (Berryman, 1970; Mills, 1985; Reeve, 1997; Weslien, 1994; Weslien and Regnander, 1992), and could be involved in the outbreak cycles seen in two bark beetle species (Berryman, 1970; Turchin et al., 1999). These results suggest that clerids could be used for augmentative biological control of bark beetles, if they could be reared in sufficient numbers.

Clerids are relatively easy to rear under laboratory conditions if natural prey are available, although the cannabalistic larvae must be separately confined. A number of studies have developed rearing methods for *Thanasimus dubius* (Fabricius) (Coleoptera: Cleridae) using natural prey, such as the southern pine beetle, *Dendroctonus frontalis* Zimmermann (Coleoptera: Scolytidae), or the engraver beetle, *Ips grandicollis* (Eichhoff) (Lawson and Morgan, 1992; Mignot and Anderson, 1969; Nebeker and Purser, 1980; Nebeker et al., 1980). These methods require substantial numbers of live prey obtained by collecting from bark beetle infestations in the field or from a laboratory culture sustained using fresh pine logs. Bark beetle larvae must then be dissected from the logs to feed the predator larvae, while emerging adults are used to feed adult predators. This process is too laborious to rear these

* Corresponding author. Fax: 1-618-453-2806.
E-mail address: jreeve@zoology.siu.edu (J.D. Reeve).

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predators in sufficient numbers for practical use in augmentation, and the culture of bark beetles requires a continuous flow of live trees. Nebeker et al. (1980) found that the cowpea weevil, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae), was a useful factitious prey for adult *T. dubius*, substituting for adult bark beetles. However, total fecundity was lower for *T. dubius* fed *C. maculatus* than on natural prey (Mizell and Nebeker, 1982).

Several technical challenges must be addressed before augmentation of predatory clerids would be feasible. These include methods of separately confining and feeding large numbers of larval predators, artificial diets or factitious prey for the larvae and adults that eliminate the need for bark beetles in the rearing process, and methods of packaging and releasing cultured predators in the field (King, 1993; Van Driesche and Bellows, 1996). We address here the problem of feeding larval clerids, through the development of a chemically-defined artificial diet devoid of insect components (a meridic diet). Two methods of presenting the diet and rearing are examined, and the performance of predators cultured on the diet and natural prey compared. We also reared *T. dubius* for five generations in the laboratory using the larval diet and *C. maculatus*, and then compared the prey preferences of cultured versus wild predators.

2. Materials and methods

2.1. Diet Preparation

We developed a meridic artificial diet that was a combination of chemically defined substances and various foodstuffs. The composition of this diet resembles the nutritional chemical composition of *I. grandicollis* larvae, after these larvae were chemically analyzed by high performance liquid chromatography and gas chromatography (M.G. Rojas and Juan A. Morales-Ramos, unpublished data). The diet is composed of a chemically defined portion (Table 1) and supplements (Table 2). The list of ingredients presented in Tables 1 and 2 represent the final composition in mg per 100 ml of diet.

The first step in preparing the diet was to make a water-soluble vitamin solution. The ingredients of the vitamin solution are listed in Table 1, and is the same solution as discussed in Rojas et al. (2000). The next step was to prepare a free amino acid solution, as reported by Rojas et al. (1996). This solution was prepared by adding 40-fold the amino acid quantities presented in Table 1 to 55 ml of distilled water. Olive oil, canola oil, lauryl sulfate neutralized to pH 7, with ~0.6 ml of 6M KOH were added to the amino acid solution (Rojas et al., 2000). After the amino acid and oil solution was autoclaved, glucose and vitamin solutions were added (Rojas et al., 2000). This resulting solution was also neutralized to pH 7, with 0.2 ml of 6 M KOH. An 8.5 ml portion of this solution was added to the ingredients as listed in Tables 1 and 2. The fat-soluble vitamins, esterols and cod liver oil were dissolved in the lauryl sulfate solution and added to the final mixture as reported by Rojas et al. (2000).

Table 1
Chemically defined portion of artificial diet in mg per 100 ml of diet

Chemicals	Amount (mg)
Vitamins	
Biotin	0.0040
Ca panthothenate	0.1415
Choline chloride	33.6806
Folic acid	0.4715
Inositol	2.1050
Nicotinamide	0.3368
Piridoxine	0.0236
Riboflavin	0.2863
Thiamine	0.0337
Vitamin B12	0.0084
Vitamin A	53.5905
Vitamin C	107.1810
Vitamin E	53.5905
Free amino acids	
Histidine	8.75
Glutamine	7.75
Cysteine	2.75
Lysine	5.50
Tryptophan	10.00
Proline	17.25
Tyrosine	18.75
Valine	2.87
Alanine	10.25
Lipids	
Cholesterol	214.36
Ergosterol	214.36
Carbohydrates	
Glucose	377.50
Sucrose	4287.25
Other	
Lauryl sulphate	214.36
6 M KOH	0.35

The animal components such as veal and veal gravy, egg white, and potted meat were ground and mixed to a paste using a hand-held food processor. The egg whites were partially cooked before adding to the diet. The final mixture had a semi-liquid texture approaching the consistency of a granular paste.

2.2. Rearing on artificial diet

Larval *T. dubius* were reared on the artificial diet using two methods. Method I used 50 cm Gelman sterile petri dishes lined with a single sheet of Kimwipe tissue (Kimberly-Clark, Roswell, GA). At the beginning of the

Table 2
List of natural products as components of artificial diet in mg per 100 ml of diet

Supplement	Amount (mg)
Oils	
Canola oil	0.15 ml
Olive oil	0.15 ml
Cod liver oil	321.54
Lecithin	1607.71
Vegetable protein	
Soy bean hydrolysate	3429.80
Yeast hydrolysate	5359.05
Animal protein	
Casein hydrolysate	3429.80
Infant formula[a]	5359.05
Egg white[b]	16077.17
Egg yolk[c]	16077.17
Veal and veal gravy[d]	26795.28
Potted meat[e]	6430.87

[a] Enfamil Nutramigen (Mead, Johnson, Evansville, IN).
[b] Semi-cooked.
[c] Raw.
[d] Gerber 2nd foods (Gerber Products, Fremont, MI).
[e] Armour potted meat food product (The Dial Corp., Scottsdale, AZ).

experiment, a single newly-hatched larva was added to each dish and the diet presented as follows. Approximately 50 μl of diet was placed on a 6×1 cm strip of stretched Parafilm M (P 7668, Sigma, St. Louis, MO), and then the film folded in half to enclose the diet droplet. Sterile distilled water was provided on a soaked 0.5 cm cube of household cellulose sponge. The diet was changed and the water renewed 6–7 times per week. After the larvae reached the prepupal stage, signified by a change in color from pink to purple, we added a $3 \times 2 \times 1$ cm chunk of sterilized pine bark to the dish as a pupation site. The bark was obtained from trees previously colonized by bark beetles, and 1–2 small holes were drilled in the bark to serve as entry points for the larvae. The developmental time to each stage was recorded during the experiment as well as the number of larvae surviving to the prepupal and adult stage. The insects were kept in an environmental chamber set for 23 °C and with a 12:12 (L:D) h photoperiod. Humidity was maintained at roughly 70% RH by adding a small bucket of water to the chamber. A total of 141 larvae were reared using this method. After individuals reached the adult stage, they were used in an experiment that measured adult performance (see below). Their elytral length (base to tip) was also measured.

Method II was devised to reduce the labor involved in rearing the larvae. It used DuPont insect rearing trays (#9073) and lids (#9072) obtained from Bio-Serv (Frenchtown, NJ) with each tray containing 16 wells. The wells were filled to a depth of approximately 0.8 cm with coarsely ground pine bark, sterilized in an autoclave. A single larva was added to each well at the beginning of the experiment. The diet was presented in Parafilm capsules formed using a 96-well tissue culture plate (R 2508, Sigma, St. Louis, MO). A sheet of unstretched Parafilm was first placed over the plate and sprayed with 70% ethanol. Indentations were then formed in the Parafilm by applying brief pressure with a cotton swab to each well. After the ethanol solution had evaporated each well was filled with approximately 50 μl of diet. A sheet of stretched Parafilm was then laid over the plate, sprayed with ethanol, and rubbed with a test tube to seal the two Parafilm layers. The 70% ethanol served to sterilize the Parafilm surfaces and acted as a lubricant in forming the wells and sealing. Similar capsules are described in Carpenter and Greany (1998) and Rojas et al. (2000).

Under Method II, a new diet capsule was presented to each larva three times a week. Sterile distilled water was provided by lightly misting each tray three times a week using a spray bottle (approximately 0.4 ml per tray). A $3 \times 2 \times 1$ cm piece of sterilized pine bark was added when each larva reached the prepupal stage. The insects were held under the same environmental conditions as Method I. Four consecutive generations were reared using Method II and the artificial diet, from 96 to 384 larvae were reared per generation. Developmental times were not recorded for this rearing method, only the number of larvae surviving to the prepupal and then adult stage. A fifth generation was reared for studies that examined prey preference (see below).

Adult predators reared using Method II were held in 20 cm high × 20 cm diam. clear plastic containers, each containing several folded booklets of brown paper towel held closed using a paper clip (Nebeker et al., 1980), to provide a hiding place and oviposition site. The adults were fed using the factitious prey *C. maculatus*, recommended by Nebeker et al. (1980) as a suitable substitute for natural prey. Water was provided on a $2 \times 2 \times 0.5$ cm piece of cellulose sponge. Prey and water were renewed three times a week, and the number of eggs laid recorded.

Laboratory rearing of *T. dubius* was initiated using eggs obtained from approximately 30 wild individuals collected using multiple funnel traps (Lindgren, 1983). The traps were baited with frontalin, the aggregation pheromone of *D. frontalis*, and steam-distilled turpentine derived from southern pine species, a combination of chemicals highly attractive to *T. dubius* adults (Billings, 1985; Vité and Williamson, 1970).

2.3. Performance of adults reared on diet vs. wild adults

Ideally, we would compare the performance of adults reared on the artificial diet with adults reared on natural prey, using identical methods except for the food source. This would require a large bark beetle culture, or the presence of nearby bark beetle infestations from which

immature stages could be harvested. We had neither of these resources, and instead used newly emerged adults collected from the field using baited traps. Trapping was conducted in early October 1999 in a pine forest located in central Louisiana. The predators have a distinct second generation per year at this site (Reeve, 2000) and trapping in October would catch newly emerged individuals from this generation. The condition of the beetles trapped also suggested they were newly emerged individuals. A drawback of this approach is that it provides no data on survival and development times of immature *T. dubius* feeding on natural prey, but there is ample information of this type in the literature, especially in a large scale rearing study conducted by Lawson and Morgan (1992). The use of wild adults does permit a comparison of laboratory-reared predators with organisms reared under fully natural conditions.

We measured the performance of adult female *T. dubius* in small arenas, consisting of a 100 cm plastic petri dish lined with a circular piece of filter paper. A folded 6×6 piece of brown paper towel, held shut using a paper clip, was added to each dish to provide an oviposition site and hiding place. At the beginning of the experiment, a single male–female pair was placed in each dish and provided with 20 live prey, an amount that should maintain them near satiation (Turnbow and Franklin, 1980; Turnbow et al., 1978). The number of prey eaten and eggs laid were recorded three times a week and the prey replenished each time. Two sources of prey were used, the natural prey *I. grandicollis* and the factitious prey *C. maculatus*. Adults of *I. grandicollis* were obtained from a small laboratory culture maintained on cut pine logs. We measured the performance of adult predators reared using the artificial diet with Method I, then fed with either *I. grandicollis* or *C. maculatus* as adults, and we compare them with wild predators fed *C. maculatus*. One-way ANOVA was used to compare the fecundity, longevity, total prey consumption, and elytral length across these three experimental groups. Tukey's honestly significant difference (HSD) test was used for multiple comparisons of the means for each group (Sokal and Rohlf, 1995). We also used analysis of covariance to test for performance differences among the groups while controlling for body size effects, with elytral length the covariate. We tested for equality of slopes across groups before the analysis of covariance (Sokal and Rohlf, 1995).

2.4. Measurement of prey preference

We conducted an experiment to determine if long-term rearing of *T. dubius* in the laboratory had an effect on their preference for natural vs. factitious prey. We compared the preference of adults from the fifth gener-

Table 3
Summary of survival rates, sex ratio, and mean duration of life stages (±SE) for *T. dubius* reared on artificial diet using Method I (see text) vs. natural prey

Biological parameters	*n*	Artificial diet	Natural prey
Survival to prepupa	141	68.1%	45.5%
Survival to adult	141	48.2%	32.3%
Sex ratio (% ♀)	60	50.0%	46.5%
Larval–prepupal duration (days)	96	31.4 ± 0.3	41.9 ± 0.6
Prepupal–adult duration (days)	68	44.1 ± 3.3	56.4 ± 1.0
Adult longevity when fed *I. grandicollis* (days)	11	64.2 ± 6.3	50.1 ± 7.1
Adult longevity when fed *C. maculatus* (days)	11	91.7 ± 10.8	—

Data for natural prey were extracted from Lawson and Morgan (1992), who used *I. grandicollis* as the prey species in rearing both larval and adult *T. dubius*. Two prey species were used to feed adult *T. dubius* reared on the artificial diet, *I. grandicollis* and *C. maculatus*. Sample sizes shown are for the artificial diet studies.

Table 4
Performance of adult female *T. dubius* (mean ± SE) reared on artificial diet using Method I vs. wild individuals collected from the field

	Artificial diet				Wild		ANOVA	
	n	Adults fed *I. grandicollis*	*n*	Adults fed *C. maculatus*	*n*	Adults fed *C. maculatus*	*F*	*P*
Fecundity (total eggs)	11	200.5 ± 35.1 a	11	224.3 ± 40.7 a	19	251.6 ± 30.2 a	0.57	0.5723
Adult longevity (days)	11	64.2 ± 6.3 a	11	91.7 ± 10.8 ab	19	127.4 ± 11.5 b	4.98	0.0120[a]
Prey eaten per pair	11	302.2 ± 37.2 a	11	130.2 ± 17.4 b	19	201.5 ± 17.2 b	10.92	0.0002
Elytral length (mm)	10	5.14 ± 0.09 a	10	5.08 ± 0.11 a	19	5.22 ± 0.07 a	0.61	0.5503
R_0	—	43.5	—	48.7	—	—	—	—

Two prey species were used to feed adult *T. dubius* reared on the artificial diet, *I. grandicollis* and *C. maculatus*. One-way ANOVA was used to compare the three groups, and means followed by different letters are significantly different at the $\alpha = 0.05$ level (see text for details). R_0 was calculated for clerids reared on artificial diet, using values from Table 3 and assuming 90% egg viability (Lawson and Morgan, 1992). The formula used was R_0 = mean fecundity × egg viability × survival to adult × sex ratio.

[a] A log transformation was applied to equalize the variance across groups.

ation of individuals reared using Method II with adults collected from the wild. Experimental arenas were established as in the performance experiments, with a single male–female pair per container, and provided 10 adult *I. grandicollis* and 10 adult *C. maculatus* as prey. Ten pairs of laboratory-reared and wild individuals were established. The number of prey eaten and eggs laid were recorded every other day for 6 d, and any eaten or dead prey replaced at that time. We used the preference index α as our measure of prey preference (Chesson, 1983), based on the last day of observations in the experiment. The last day was chosen because it allowed some time for the predators to acclimate to laboratory conditions and the two prey species, but in any event there appeared to be little shift in behavior through time. Preference for *I. grandicollis* was calculated using the formula

$$\alpha_{Ig} = \frac{\ln((n_{Ig} - r_{Ig})/n_{Ig})}{\ln((n_{Ig} - r_{Ig})/n_{Ig}) + \ln((n_{Cm} - r_{Cm})/n_{Cm})}, \quad (1)$$

where n_{Ig} and n_{Cm} are the initial numbers of *I. grandicollis* and *C. maculatus*, and r_{Ig} and r_{Cm} are the number of prey eaten (Chesson, 1983). This version of α allows for the depletion of prey during the experiment. We compared α across laboratory and wild *T. dubius* using the non-parametric Mann–Whitney rank test (Conover, 1999).

The total percent of prey consumed was used to gauge overall predator voracity. We compared this quantity across laboratory and wild *T. dubius* using a two-sample *t* test, after applying the arcsine square root transformation.

3. Results

Survival rates and the duration of various life history stages are reported in Table 3, which compares *T. dubius* reared on the artificial diet using Method I with a large scale rearing study using *I. grandicollis*, a natural prey species for this predator (Lawson and Morgan, 1992). Survival rates appeared to be higher on the artificial diet, while the larval–prepupal and prepupal–adult developmental times were shorter, even though rearing temperatures were similar in the two studies. Adult longevity also appeared to be longer for individuals reared on the diet, regardless of the prey fed to the adults.

Table 4 compares the performance of adult *T. dubius* reared using Method I with newly-emerged wild adults collected from the field. There were no significant differences in either fecundity or size across the three treatment groups, although wild adults appeared to be somewhat larger and more fecund. Longevity and the number of prey eaten were significantly different across groups. Adults reared on the artificial diet and then fed *I. grandicollis* were shorter-lived than the other two groups, especially the wild individuals (Table 4). They also consumed more total prey during the course of the experiment, likely because *I. grandicollis* are smaller prey than *C. maculatus*.

Analysis of covariance revealed essentially the same treatment effects as in Table 3, after controlling for effects of elytral length. Fecundity was similar across the

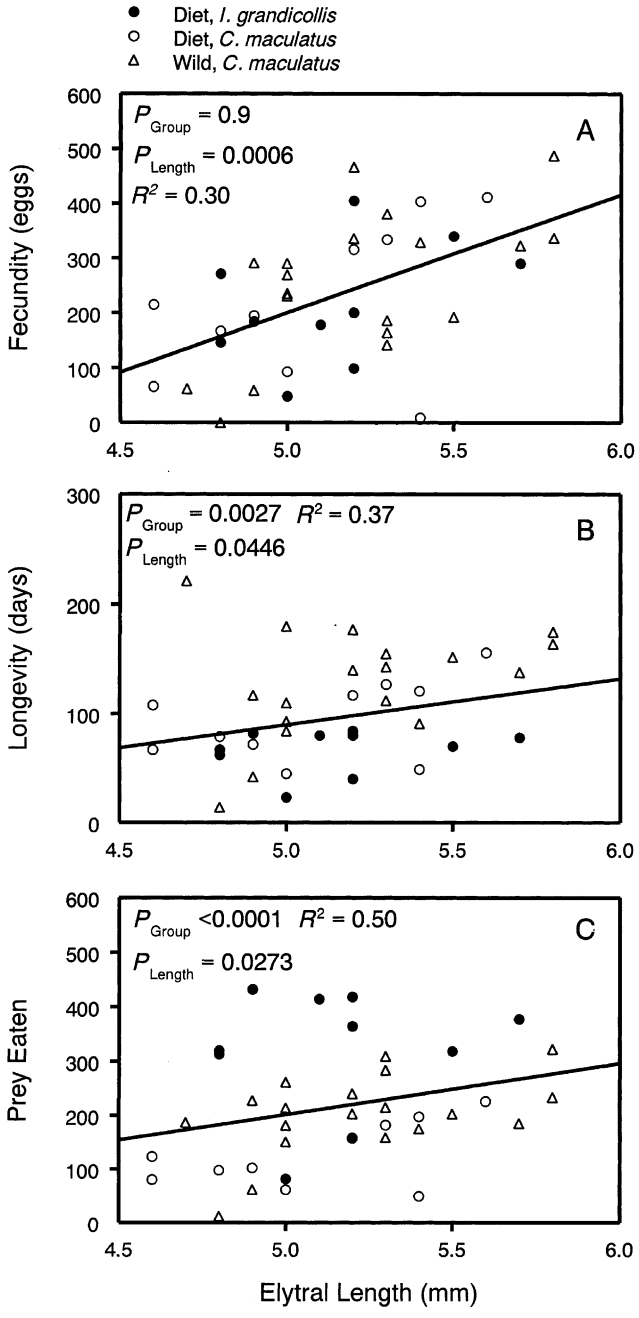

Fig. 1. Fecundity (A), longevity (B), and total prey eaten (C) vs. elytra length for predators reared on diet and then fed either *I. grandicollis* or *C. maculatus* as adults, and for wild adults fed *C. maculatus* (see legend). *P* and R^2 values are from an analysis of covariance comparing the three experimental groups (see text for details). The line in each graph indicates the slope of the relationship between these performance variables and elytral length.

Table 5
Summary of survival rates, total eggs laid, and R_0 for *T. dubius* reared using artificial diet and Method II

Biological parameters	Generation				Mean ± SE
	I	II	III	IV	
Initial larvae	96	384	256	256	—
Survival to prepupa	70.8%	44.8%	74.2%	78.1%	67.0 ± 7.5%
Survival to adult	61.5%	36.2%	61.7%	63.7%	55.8 ± 6.5%
Sex ratio (% ♀)	43.2%	42.2%	53.0%	53.9%	48.1 ± 3.1%
Total eggs laid	2876	6314	10,870	14,818	—
Elytral length (♀) (mm)	4.71	4.71	4.90	4.91	4.81 ± 0.06
R_0	27.0	14.8	38.3	52.1	33.1 ± 8.0

R_0 was calculated assuming 90% egg viability (Lawson and Morgan, 1992) and using the formula $R_0 = 0.9 \times$ total eggs laid/initial larvae. Also shown are average elytral lengths of female predators for each generation. Mean values across all four generations are shown in the last column.

treatment groups, but longevity and the number of prey eaten were significantly different (Fig. 1). There was a significant effect of adult size as indicated by elytral length on all three performance variables, with larger females laying more eggs, living longer, and consuming more prey.

Table 5 shows survival rates, total number of eggs laid, and R_0 values for predators reared using Method II and the artificial diet. Survival rates were similar to Method I, but elytral lengths were smaller, especially in the first two generations. Excessive amounts of water may have been used during the second generation and could explain its poor performance. A one-way ANOVA was used to compare the elytral length of female *T. dubius* across Method I, the four generations reared using Method II, and newly-emerged wild individuals. The overall test comparing these groups was highly significant ($F = 7.66$, df $= 5$, 235, $P < 0.0001$), but group effects explained only a small proportion of the total variability ($R^2 = 0.14$). Overall, adult female predators reared using Method I were about 2% smaller than wild individuals (5.11 vs. 5.22 mm), while the third and fourth generations using Method II (4.90 mm) were 6% smaller. The first two generations using Method II were about 10% smaller than wild individuals (4.71 vs. 5.22 mm). R_0 values were similar across the two rearing methods, especially the last two generations reared using Method II (see Tables 3 and 4).

No significant difference was observed in prey preference between the fifth generation of *T. dubius* reared using Method II and wild individuals ($\alpha = 0.871$ vs. 0.931, $P = 0.43$, Mann–Whitney rank test). Individuals reared on the artificial diet were significantly more voracious than wild individuals, consuming a higher percentage of the total prey provided (38.8% vs. 28.0%, $P = 0.0089$, two-sample t test).

4. Discussion

The artificial diet appears to be an effective substitute for natural prey in rearing larval *T. dubius*. Survival rates, fecundities, and R_0 values appear to match or exceed those found in previous studies that reared this predator on *Ips* prey. For example, we calculate that Lawson and Morgan (1992) obtained $R_0 \approx 20$ using *I. grandicollis* as prey, about half the value obtained using the artificial diet and *C. maculatus*. Mignot and Anderson (1969) obtained an even lower value using various *Ips* species as prey ($R_0 \approx 15$). It is possible our study underestimates the difference in performance between wild and diet-reared adults, however, because the wild adults collected from the field were of indefinite age, although most were probably newly emerged. Mizell and Nebeker (1982) also obtained substantially higher fecundities in a study that used adult predators emerging from material infested by *D. frontalis*. Fecundities were especially high when the predators were fed *D. frontalis* as adults vs. alternative prey. We speculate that *D. frontalis* may be the optimal prey species for *T. dubius* in the southern portion of its range, producing adult predators that are more fecund than with other prey species.

No difference in prey preference was found between wild and diet-reared individuals, even after five generations in the laboratory. While this is encouraging, further research is needed on the response of diet-reared predators to bark beetle pheromones. Clerid beetles locate their prey using these pheromones (Raffa, 2001; Vité and Williamson, 1970; Wood, 1982), and could potentially lose this ability if reared for long periods of time under artificial conditions. Repeated infusions of wild individuals into laboratory cultures would presumably reduce this problem.

There was no substantial difference in the quality of predators reared using Method I vs. II, and Method II required less time because the larvae were fed only three times a week. We could not reduce the number of feedings any further because of concerns about spoilage (the diet contains no preservatives and is non-sterile). Further improvements in rearing methods would require an increase in the shelf-life of the diet, or more efficient ways of individually feeding the larvae.

Sufficient numbers of predators could be generated using the techniques in this paper for small-scale field trials of augmentation. For example, we estimate that several hundred *T. dubius* eggs per meter of infested bole would generate densities at the high end of the natural scale, based on daily oviposition rates and surface densities of predators (Reeve, 1997; Turnbow et al., 1978). Given the number of eggs produced using Method II (Table 5), it should be possible to elevate populations of larval predators at least within caged areas of the tree, if not entire trees. However, augmentation using adult *T. dubius* would require substantial improvements in our rearing techniques. It would not be possible to treat individual trees using adult predators, because these are highly mobile (Cronin et al., 2000). Instead, we would need to treat entire bark beetle infestations, and therefore much larger numbers of predators would be required.

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